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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07C 317/36, C07D 219/10, A61K 31/135

A1

(11) International Publication Number: WO 97/13748

(43) International Publication Date: 17 April 1997 (17.04.97)

(21) International Application Number: PCT/KR96/00173

(22) International Filing Date: 9 October 1996 (09.10.96)

(30) Priority Data:
1995-34486 9 October 1995 (09.10.95) KR

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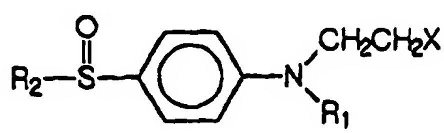
(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

**Published**
*With international search report.*

(54) Title: SULFOXIDE DERIVATIVES OF NITROGEN-MUSTARD AND ANTICANCER AGENT CONTAINING THE SAME

(57) Abstract

According to the present invention, are provided sulfoxide derivatives of nitrogen-mustard represented by general formula (I), wherein $R_1$ represents a hydrogen, or haloethyl; $R_2$ represents a lower alkyl or phenyl group substituted or non-substituted; and X represents a halogen atom. Provided that $R_1$ is a chloroethyl and X is a chlorine, $R_2$ does not represent methyl group; and an anticancer agent comprising the same as a prodrug. The sulfoxide derivatives according to the present invention convert to a sulfide in a hypoxic condition to show cytotoxicity, so that it is very useful as an anticancer agent selectively functions on a solid cancer which is in a hypoxic condition.

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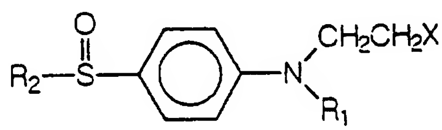
## TITLE OF INVENTION

## SULFOXIDE DERIVATIVES OF NITROGEN-MUSTARD AND ANTICANCER AGENT CONTAINING THE SAME

## TECHNICAL FIELD

The present invention relates to sulfoxide derivatives of nitrogen-mustard which are represented by a general formula:



(I)

wherein, $R_1$ represents a hydrogen, or haloethyl; $R_2$ represents a lower alkyl or phenyl group substituted or non-substituted; and X represents a halogen atom. Provided that $R_1$ is a chloroethyl and X is a chlorine, $R_2$ does not represent methyl group;

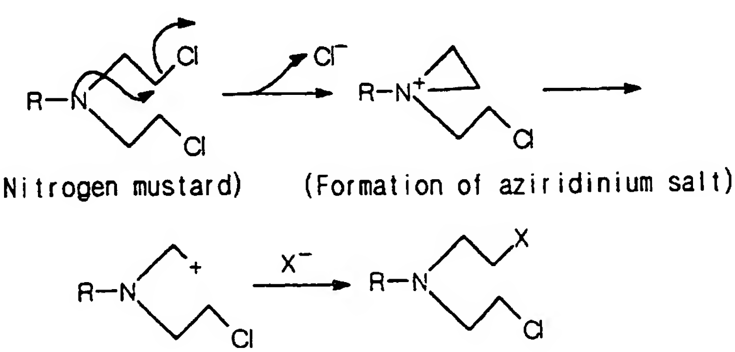
and pharmaceutically acceptable salts thereof, and their use as an anticancer agent.

## BACKGROUND ART

Despite improvements in chemotherapy for cancer in the past few decades, due care have been required in the clinical application of conventional anticancer agents in many cases due to their non-specific selectivity, including undesirable side effects. With their physiological properties (e.g., cellular heter ogeneity and inadequate blood flow in the local tumor site), solid tumors have shown little responsiveness on chemotherapy and are also resistant to radiotherapy (Warren, 1978; Poste, 1986).

Now that said solid tumor cells have been reported to be in localized hypoxia, nutrient deprivation, and low pH (Kennedy et al., 1980), some selective-specific drugs on target sites have been suggested in consideration of said tumor's specific physiological and environmental factors (Denny and Wilson, 1986; Stratford et al., 1986; Sartorelli, 1988; Wilson et al., 1989a and 1989b; Denny et al., 1990). In view of these situations, sulfoxide derivatives have been the remarkable prominent. Said compound is converted into sulfide, a physiologically active metabolite, by redox enzyme present in the mammal tissues, especially under hypoxic conditions.

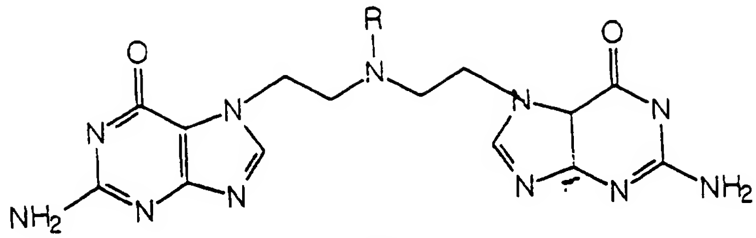
In the meanwhile, nitrogen mustard forms aziridinium ion based upon the following reaction formula (A), and combines with a DNA base by alkylation reaction to form the structure as illustrated in Example (B). Since nitrogen mustard can optionally attack any position of oxygen or nitrogen having lone pair electrons of DNA base molecule such as cytocine, guanine, thymine and adenine, it demonstrates a strong toxicity to cells. Further, with its non-selectivity property in distinguishing tumor cells from normal cells, nitrogen mustard shows a strong toxicity to normal cells.




(Alkylation)

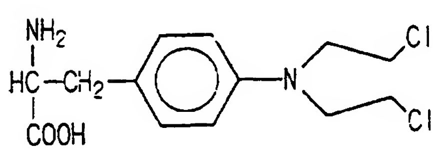
(A)

[In the formula, X represents DNA base.]

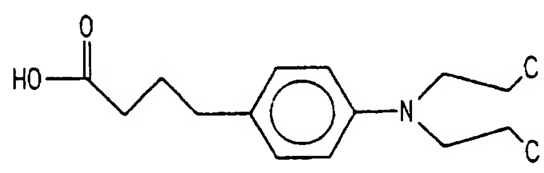


(B)

In order to alleviate the toxicity of nitrogen mustard, attempts have been made to delocalize the lone pair electrons of nitrogen. Among these, melphalan is an antineoplastic agent having phenylalanine substituent on R group of nitrogen mustard. The active form of this compound is delivered to the cells in a transport system of amino acid, which is indicated for the treatment of osteoma, ovarian cancer or mastocarcinoma by the therapy of a single agent or with concurrent administration. However, some side effects associated with the overdose of melphalan are myelosuppression, gastrointestinal disturbances, and hypersensitivity; it has relatively low selectivity with negligible effects. Chlorambusil with a similar structure to melphalan has been used for a long term treatment of leukemia but its high toxicity has led to some side-effects such as myelosuppression, nausea, hepatic disorder and hepatica tocixity.



melphalan

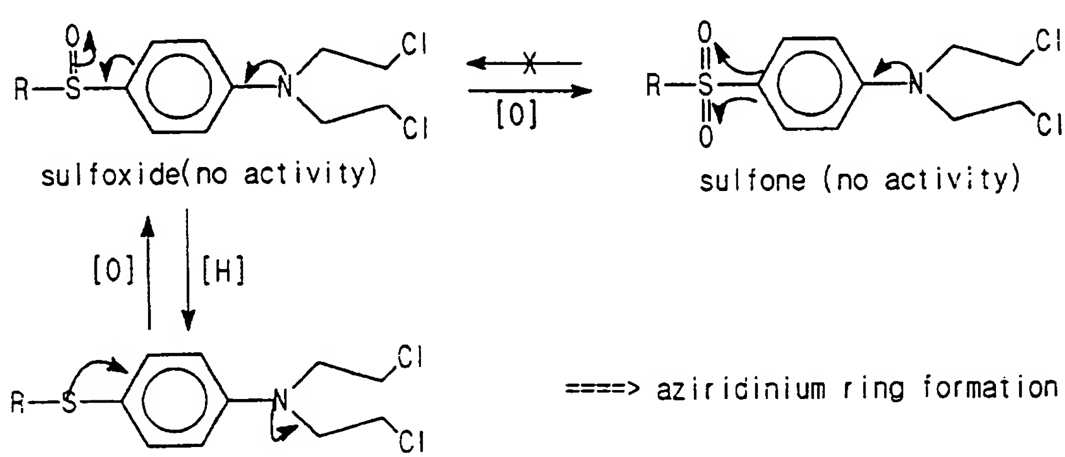


chlorambusil

To be free from the aforementioned disadvantages the conventional arts face, the present inventors have

endeavored to conduct the research and development of the compounds having strong activity and high selectivity to tumor cells thus, designing and synthesizing sulfoxide derived from nitrogen mustard, which is converted to the corresponding sulfide, a physiologically active metabolite as mentioned above, to complete this invention.

In general, sulfoxide derivatives of nitrogen mustard do not form a bond with a DNA base by aziridine ring formation as mentioned above, so that it has no antineoplastic activity. Also, sulfone derivatives, metabolites of the sulfoxide derivatives, have no antineoplastic activity in a normal state because of the same reason. However, sulfoxide derivatives are metabolized to a sulfide derivatives under anaerobic (hypoxic) conditions, and the sulfide group readily go through aziridine ring formation, whereby showing very selective cytotoxicity. As the fact that the inner tumor cells, especially a solid cancer tissue has extremely hypoxic condition is well known, the sulfoxide derivatives having the above-described property are very useful for the treatment of solid cancers.




sulfoxide(no activity)

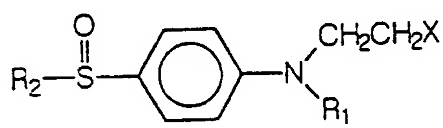
sulfone (no activity)

sulfide (high activity)

In 1992, p-(methylsulfinyl)phenyl nitrogen mustard as a sulfoxide prodrug of an anti-cancer agent, which is converted to a sulfide, under hypoxic conditions, designed to specifically attack the tumor cells, has been developed by the inventor of the present invention. However, the compound has insufficient cytotoxicity (specific action to the tumor cells), so that an anticancer agent having more selectivity and more excellent effect.

## DISCLOSURE OF THE INVENTION

As a result of intensive study to develop the prodrug of an anti-cancer medicine having excellent selectivity to tumor cells, the present inventor unexpectedly found that the sulfoxide derivatives of nitrogen mustard represented by general formula (I) can fulfill these requirements.



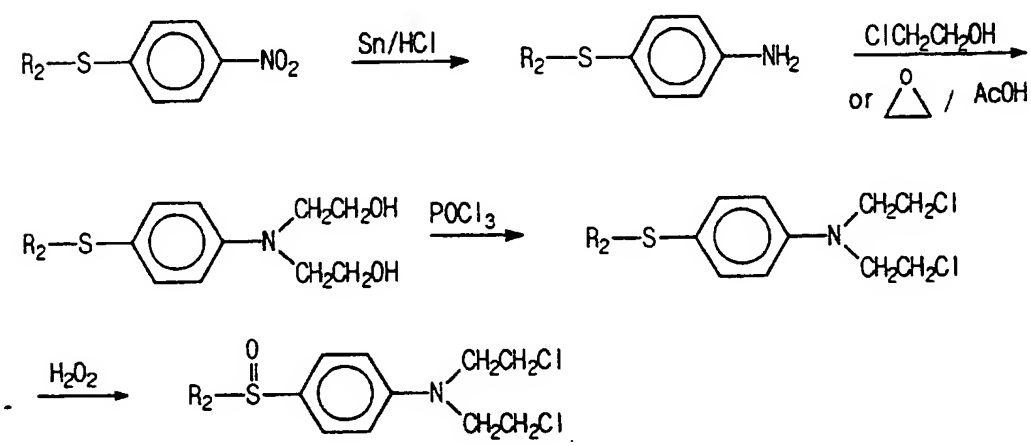
(I)

In the formula, $R_1$, $R_2$ and X are defined as above.

Among the compounds, p-(n-propylsulfinyl)-N,N-bis(2-chloroethyl)aniline, p-(phenylsulfinyl)-N,N-bis(2-chloroethyl)aniline, 4,4′-N,N,N′,N′-tetrakis(2-chloroethyl)dianiline sulfoxide, 4,4′-N,N′-bis(2-chloroethyl)dianiline sulfoxide and 4-N,N-bis(chloroethyl)amino-4′-(9-acridinyl)aminodiphenyl sulfoxide are mentioned as desirable compounds. The compounds of the present invention can be synthesized by the use of known methods. For example, the compounds, wherein $R_2$ is lower alkyl or phenyl group substituted or non-substituted and $R_1$ is haloethyl, can be prepared by

reducing the nitro group at p-position to introduce an amino group; reacting with 2-chloroethanol or ethylene oxide/ acetone to introduce a hydroxyethyl group at N-position; reacting the product with halogenating agent; and then oxidizing the resultant sulfide group, as illustrated in Scheme I.

Scheme I

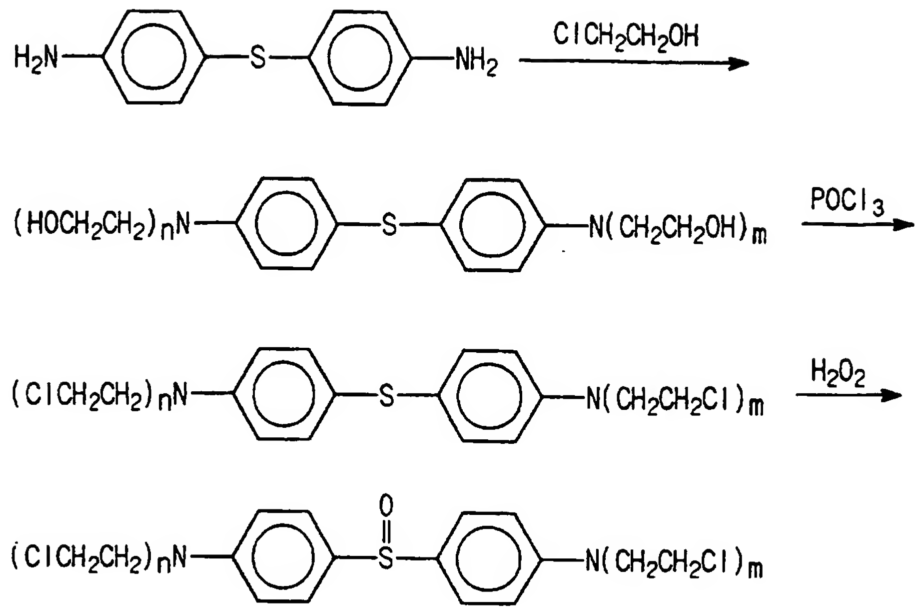



(In the formula, $R_2$ is lower alkyl or phenyl group substituted or non-substituted.)

The compounds, wherein $R_2$ is lower alkyl or phenyl group substituted or non-substituted and $R_1$ is H, can be systhesized by concentration control of $ClCH_2CH_2OH$ used at the second step of the above scheme I.

The compounds wherein $R_2$ is an aniline group having one or more lower alkyl group with halogen substituent at N-position can be obtained by firstly preparing 4,4'-diaminodiphenyl sulfide; introducing one or more hydroxyethyl group to the amino group of the compound; halogenating the introduced hydroxy group; and oxidizing the sulfide group, as illustrated in Scheme II.

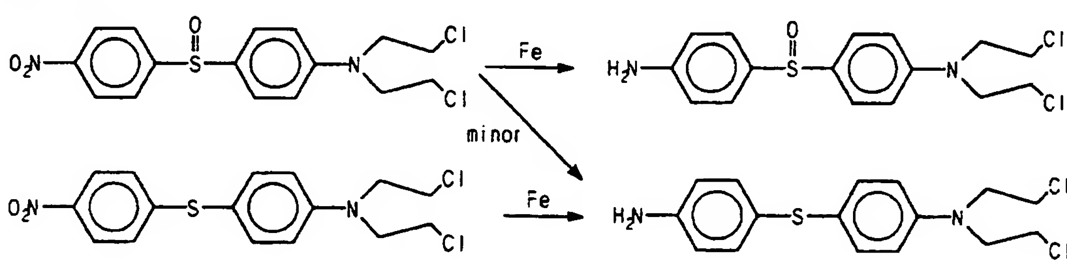
Scheme II



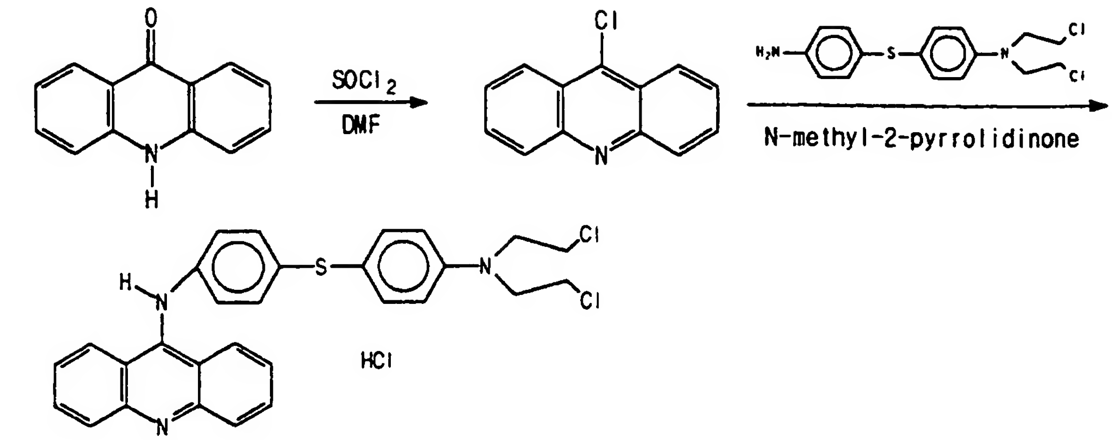
(In the formula, n and m are 0, 1, 2, but on the other hand n and m can not be 0 simultaneously)

Depending upon the features of the substituent included in $R_2$ group, various derivatives may be synthesized as shown, for example, in the following reaction schemes :

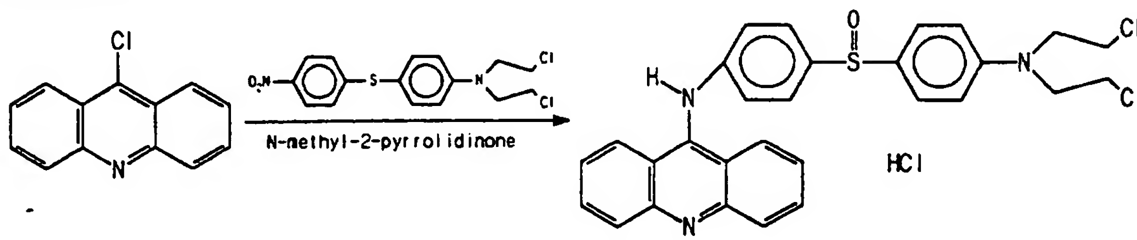
Scheme III



Scheme IV




Scheme V




## BRIEF DESCRIPTION OF THE DRAWINGS

Figs. 1 - 4 are graphs of plotting the survival rate of V-79 cells under normal condition or hypoxic condition with regard to the concentration of medicines, when the compound of Example 5, 10, 14 or 17 is administered (Experimental Example 1).

Figs. 5 and 6 show the result of the test for tumor inhibition in vivo (Experimental Example 2).

## BEST MODE FOR CARRYING OUT THE INVENTION

Here-in-after, the present invention will be described in detail by referring to the following Examples and Experimental Examples. However, it should be noted that

the present invention is not restricted to these Examples.

Example 1 : p-(n-Propylthio)nitrobenzene

1-Bromopropane (7.5 ml, 8.24 g, 83 mmol) was added with stirring to a solution of p-nitrothiophenol (10.70 g, 69 mmol) in 50 ml of ethyl alcohol. To the clear orange-color solution, 40% NaOH solution (6.9 ml, 69 mmol) was added and the solution was heated under reflux for 40 min. The clear orange solution was cooled, and ice water was added thereto. The mixture was extracted with methylene chloride (4 x 100 ml). The combined organic layer was dried over anhydrous $Na_2SO_4$ and evaporated to give 14.92 g of brown oil which contained 4 substances detected by UV after TLC operation. This brown oil was applied to a silica gel column (40 $\mu$m; 5 x 17 cm) packed dry. Then it was wet and eluted with hexane/EtOAc (4:1) under a positive pressure (15 psi). The corresponding fractions were collected, and the solvent was removed by evaporation to give 11.20 g (yield: 86.3%) of pure compound as orange crystalline solid.

m.p. = 102-103 °C

TLC : $R_f$ = 0.71 in hexane/EtOAc (4:1)

$^1H$ NMR ($CDCl_3$) $\delta$ (ppm)

7.32-7.60 & 8.15-8.44 (dd, 4H, $-C_6H_4-$), 3.01-3.33 (t, 2H, $-CH_2S-$), 1.66-2.19 (m, 2H, $CH_2$), 1.08-1.50 (t, 3H, $CH_3$)

Example 2 : p-(n-Propylthio)aniline

To a boiling suspension of p-(n-propylthio)nitro benzene (11.20 g, 67 mmol) suspended in a solution of 37% HCl (35 ml) and water (30 ml), tin turnings (11.0 g) were added upon which the suspension became a clear light brown solution. The solution was heated under reflux with vigorous stirring for 15 min. Activated charcoal (0.1 g) was added and the mixture was allowed to continue refluxing

for an additional 15 min. The mixture was filtered hot and the filtrate was cooled and diluted with cold water (100 ml). Aqueous NaOH (40%) was added till the mixture became strongly alkaline. The resulting gray product was filtered. The filtered product was suspended in boiling ethyl alcohol (60 ml), and charcoal was added thereto. The suspension was heated under reflux for 30 min. The mixture was filtered hot, and the filtrate was quickly added to ice cold water (500 ml) causing the formation of a white precipitate. The mixture was then filtered and dried to give 10.1 g of crude white powder which contained 3 substances detected by UV after TLC operation. This product was dissolved in hexane/EtOAc (4:1) (25 ml) and applied to a silica gel column (40 $\mu$m; 5 x 17 cm) packed dry. Then it was wet and eluted with the same solvent. The collected corresponding fractions were dried over anhydrous sodium sulfate and evaporated to give 8.13 g (yield: 85.6%) of pure compound as yellow oil.

TLC : $R_f$ = 0.37 [hexane/ ethyl acetate (4:1)]

$^1H$ NMR ($CDCl_3$) $\delta$ (ppm)

6.55-6.82 & 7.19-7.50 (dd, 4H, $-C_4H_6-$), 3.58 (s, 2H, $NH_2$), 2.68-3.08 (t, 2H, $CH_2S-$), 1.32-1.93 (m, 2H, $CH_2$), 0.85-1.30 (t, 3H, $CH_3$)

<u>Example 3 : p-(n-Propylthio)-N,N-bis(2-hydroxyethyl)aniline</u>

p-(n-Propylthio)aniline (8.12 g, 49 mmol) was added to a suspension of $CaCO_3$ (10.00 g, 100 mmol) in water (250 ml). The cloudy mixture was heated under reflux with vigorous stirring, protected from light, for 24 h. An additional four equivalents of 2-chloroethanol (14.3 ml, 196 mmol) and $CaCO_3$ (10.00 g, 100 mmol) were added in two(2) equivalent portions over the following 48 h. The reaction mixture was then cooled, pH (5.1) adjusted to 7.0 with 10% aqueous NaOH, and the mixture was extracted with EtOAc (4 x 200 ml). The combined organic layer was dried over anhydrous

sodium sulfate and evaporated to give 8.18 g of a brown oil. The crude material was applied to a silica gel column (40 $\mu$m, 5 x 17 cm) packed dry. Then it was wet and eluted with EtOAc/hexane (2:1) under a positive pressure (15 psi). The collected corresponding fractions were dried over anhydrous sodium sulfate and evaporated to give 3.80 g (yield: 30.6%) of the product as a pale brown oil.

TLC : $R_f$ = 0.46 [hexane/ ethyl acetate (1:3)]

$^1$H NMR ($CDCl_3$) $\delta$ (ppm)

6.38-6.73 & 7.05-7.40 (dd, 4H, $-C_6H_4-$), 3.15-3.94 [m, 10H, $-N(CH_2CH_2OH)_2$], 3.14-2.95 (t, 2H, $CH_2S-$), 1.32-1.68 (m, 2H, $-CH_2-$), 0.73-1.12 (t, 3H, $CH_3$).

Example 4 : p-(n-Propylthio)-N,N-bis(2-chloroethyl)aniline

p-(n-Propylthio)-N,N-bis(2-hydroxyethyl)aniline (3.80 g, 15 mmol) was dissolved in $POCl_3$ (9.0 ml) with stirring. The solution was heated under reflux at 100°C in a water bath for 40 min, then the hot reaction mixture was cooled to room temperature. The mixture was extracted with ethyl acetate (4 x 100 ml) and washed with 10% sodium bicarbonate solution (3 x 200 ml). The combined organic layer was dried over anhydrous sodium sulfate and evaporated to give 3.23 g of the product as a brown oil.

TLC : $R_f$ = 0.84 [hexane/ ethyl acetate (2:1)]

$^1$H NMR ($CDCl_3$) $\delta$ (ppm)

6.48-6.73 & 7.07-7.46 (dd, 4H, $-C_6H_4-$), 3.32-3.75 [m, 8H, $-N(CH_2CH_2Cl)_2$], 2.58-2.89 (t, 2H, $CH_2S-$), 1.32-1.82 (m, 2H, $-CH_2-$), 0.76-1.15 (t, 3H, $CH_3-$)

Elemental analysis $C_{13}H_{19}Cl_2NS$

Calcd. : C, 53.42; H, 6.55; N, 4.79; S, 10.97; Cl, 24.26

Found : C, 53.44; H, 6.53; N, 4.81; S, 10.87; Cl, 24.18

Example 5 : p-(n-Propylsulfinyl)-N,N-bis (2-chloroethyl) aniline

To a solution of p-(n-propylthio)-N,N-bis(2-chloroethyl)aniline (1.00 g, 3.4 mmol) in trifluoroacetic acid (5 ml), $H_2O_2$/trifluoroacetic acid (1.0 ml, 1 equivalent), prepared by adding 30% $H_2O_2$ (8.6 ml) to trifluoroacetic acid (16.4 ml), was added while stirring at 0°C. The reaction was allowed to proceed for 2h. The ice bath was removed and the reaction was allowed to continue for an additional 30 min. Trifluoroacetic acid was evaporated to give 0.78 g (yield : 74.3 %) of pure product as a light yellow solid.

TLC : $R_f$ = 0.41 [hexane/ ethyl acetate (1:3)]

$^1H$ NMR ($CDCl_3$) $\delta$ (ppm)

6.30-6.61 & 6.98-7.39 (dd, 4H, $-C_6H_4-$), 3.38-3.79 [m, 8H, $-N(CH_2CH_2Cl)_2$], 2.71-3.00 (t, 2H, $CH_2SO-$), 1.40-1.84 (m, 2H, $-CH_2-$), 0.78-1.15 (t, 3H, $CH_3-$)

Elemental analysis $C_{13}H_{19}Cl_2NSO$

Calcd. : C, 50.65; H, 6.21; N, 4.54; S, 10.40; Cl, 23.00

Found : C, 50.39; H, 6.09; N, 4.63; S, 10.47; Cl, 22.85

Example 6 : p-(4-nitrophenyl)phenyl sulfide

Thiophenol (3.0 ml, 4.73 g, 43 mmol) was added to a boiling solution of 1-bromo-4-nitrobenzene (8.70 g, 43 mmol) in ethyl alcohol (50 ml). Aqueous NaOH (40%) (3.0 ml, 43 mmol) was then added drop-wise to the solution and the solution heated under reflux for 1 h. The solution was cooled, diluted with water (200 ml), and then extracted with $CH_2Cl_2$ (4 x 150 ml). The combined organic layer was washed with 10% NaOH, dried over anhydrous $Na_2SO_4$ and evaporated to give 8.63 g (85% yield) of the product as chromatographically pure yellow solid.

m.p. : 89 - 91 °C

TLC : $R_f$ = 0.69 [hexane/ ethyl acetate (20:1)]

$^1$H NMR ($CDCl_3$) δ (ppm)

7.41 (s, 5H, $C_6H_5S$), 7.02-7.73 (dd, 4H, $C_6H_4NO_2$-)

IR (Nujol, $cm^{-1}$)

3030, 1600-1500 ($C_6H_4$), 1530, 1350 ($NO_2$)

Example 7 : p-Phenylthioaniline

With the use of p-(4-nitrophenyl)phenyl sulfide (59.7 mmol, 13.80 g) prepared in Example 6, the procedure of Example 2 was repeated to give 11.65 g of white solid powder which contained 4 substances detected by UV after TLC operation. The solid was dissolved in 30 ml of hexane/EtOAc (3:1), applied to a silica gel column (40 μm; 5 x 17 cm) packed dry. Then it was wet and eluted with hexane/EtOAc (3:1) at a positive pressure (15 psi). The collected corresponding fractions were evaporated to obtain 10.23 g (yield: 85.3%) of the product as while solid crystalline product.

m.p. : 91-93 °C

TLC : $R_f$ = 0.48 [hexane/ ethyl acetate (3:1)]

$^1$H NMR ($CDCl_3$) (ppm)

7.13 (s, 5H, $C_6H_5S$), 6.68 - 7.50 (dd, 4H, -$C_6H_4NH_2$)

Example 8 : p-(Phenylthio)-N,N-bis(2-hydroxyethyl)aniline

With the use of p-phenylthioaniline (50.0 mmol, 10.05 g) prepared in Example 7, the procedure of Example 3 was repeated to give 9.60 g of the crude product as a brown oil. The oil was applied to a silica gel column (40 μm, 5 x 17 cm) packed dry. Then it was wet and eluted with ethyl acetate / hexane (1:1) under a positive pressure (15 psi). The collected corresponding fractions were dried over anhydrous sodium sulfate and evaporated to obtain 2.26 g (yield: 16.2%) of the product as brown oil.

TLC : $R_f$ = 0.25 [hexane/ ethyl acetate (1:2)]

$^1H$ NMR ($CDCl_3$) $\delta$ (ppm)

7.19 (s, 5H, $C_6H_5S$-), 6.52-6.77 (dd, 4H, $C_6H_4$-), 3.26-4.00 [m, 10H, $N(CH_2CH_2OH)_2$]

<u>Example 9 : p-(Phenylthio)-N,N-bis(2-chloroethyl)aniline</u>

With the use of p-(phenylthio)-N,N-bis(2-hydroxyethyl)aniline (7.82 mmol, 2.26 g) prepared in Example 8, the procedure of Example 4 was repeated to give 2.11 g of the crude product as a light brown viscous oil. The crude product was applied to a silica gel column (40 μm, 2.5 x 12.5 cm) packed dry. Then it was wet and eluted with ethyl acetate / hexane (1:4) under a positive pressure (15 psi). The collected corresponding fractions were dried over anhydrous sodium sulfate and evaporated to obtain 2.03 g (yield: 80%) of the product as light brown oil.

TLC : $R_f$ = 0.69 [hexane/ ethyl acetate (4:1)], UV quenching and NBP positive

$^1H$ NMR ($CDCl_3$) $\delta$ (ppm)

7.11 (s, 5H, $C_6H_5S$-), 6.40-7.48 (dd, 4H, $C_6H_4N$-), 3.32-3.78 [m, 8H, $N(CH_2CH_2Cl)_2$]

Elemental analysis $C_{16}H_{17}Cl_2NS$

Calcd. : C, 58.90; H, 5.25; N, 4.29; S, 9.83; Cl, 21.73

Found : C, 58.77; H, 5.16; N, 4.23; S, 9.72; Cl, 21.70

<u>Example 10 : p-(Phenylsulfinyl)-N,N-bis(2-chloroethyl) aniline</u>

With the use of p-(phenylthio)-N,N-bis(2-chloroethyl)aniline (3.1 mmol, 1.00 g) prepared in Example 9, the procedure of Example 5 was repeated to give 0.84 g of the crude product as dark brown viscous oil. The crude product was applied to a silica gel column (40 μm, 2.5 x 12.5 cm) packed dry. Then it was wet and eluted with ethyl

acetate / hexane (3:1) under a positive pressure (15 psi). The collected corresponding fractions were dried over anhydrous sodium sulfate and evaporated to obtain 0.71 g (yield: 68%) of the product as an light yellow crystalline product.

m.p. : 122-124 °C

TLC : $R_f$ = 0.68 [hexane/ ethyl acetate (1:3)]

$^1H$ NMR ($CDCl_3$) δ (ppm)

7.20 (s, 5H, $C_6H_5S$-), 6.51-7.59 (dd, 4H, -$C_6H_4N$-), 3.36-3.77 [m, 8H, $N(CH_2CH_2Cl)_2$]

Elemental analysis $C_{16}H_{17}Cl_2NSO$

Calcd. : C, 56.15; H, 5.01; N, 4.09; S, 9.37; Cl, 20.72

Found : C, 56.20; H, 5.03; N, 4.04; S, 9.45; Cl, 20.64

Example 11 : 4,4'-Diaminodiphenyl sulfide

4-Amino-4'-nitrodiphenyl sulfide (10.00 g, 40 mmol) was suspended in a solution of conc. HCl (37%) (26.5 ml) and water (25 ml), and the light yellow suspension was heated to 100°C. To the boiling suspension, thus obtained tin turnings (9.00 g) were added to form a clear dark yellow solution. Additional HCl (2.0 ml) and tin turnings (1.00 g) were added and the solution was allowed to boil under reflux for 15 min. Activated charcoal (0.1 g) was added to the mixture and the reaction was allowed to boil for an additional 10 min. The mixture was filtered hot and the filtrate was allowed to cool. To the cooled filtrate, water (150 ml) and 40% aqueous NaOH was added till the solution became strongly alkaline (pH 12-13) and the resulting precipitate was filtered. The filtered grey solid product was dissolved in boiling alcohol (60 ml) and heated with activated charcoal (0.25 g) under reflux for 30 min. The mixture was filtered hot and the hot filtrate was added to ice cold water (500 ml) which resulted in the

precipitation of a white solid. The solid was filtered and dried to give 6.51 g (yield: 71.2 %) of the product as a white crystalline solid.

m.p. : 104 - 107 °C

TLC : $R_f$ = 0.50 [hexane / ethyl acetate (1:1)]

Example 12 : 4,4'-N,N,N',N'-Tetrakis(2-hydroxyethyl)dianiline sulfide

4,4'-Diaminodiphenyl sulfide (6.50 g, 30 mmol) and 2-chloroethanol (16.1 ml, 19.32 g, 240 mmol) were added to a suspension of $CaCO_3$ (12.00 g, 120 mmol) in water (200 ml). The cloudy mixture was heated under reflux with vigorous stirring, while being protected from light for 24 h. An additional 4 equivalents of 2-chloroethanol (8.0 ml, 9.66 g, 120 mmol) and $CaCO_3$ (6.00 g, 60 mmol) were added and the reaction was allowed to continue for 24 h. The reaction mixture was then cooled, and the pH (2.6) was adjusted to 7.0 with 10% NaOH. The mixture was then extracted with ethyl acetate (4 x 100 ml). The combined organic layer was dried over anhydrous sodium sulfate and evaporated to give 6.60 g of a dark brown viscous oil which contained 5 substances detected by UV after TLC operation. The crude oil was then applied to a silica gel column (40 µm, 5 x 15 cm) packed dry. Then it was wet and eluted with ethyl acetate under a positive pressure (15 psi). The collected corresponding fractions were dried over anhydrous sodium sulfate and evaporated to obtain 3.15 g (yield: 26.8%) of the product as clear viscous oil.

TLC : $R_f$ = 0.30 (ethyl acetate)

$^1H$ NMR ($CDCl_3$) $\delta$ (ppm)

6.32-6.72 & 6.90-7.30 [dd, 8H, $S(C_6H_4)2$],

3.16-3.68 [m, 20H, $N(CH_2CH_2OH)_2$]

Example 13 : 4,4'-N,N,N',N'-Tetrakis(2-chloroethyl)dianiline sulfide

With the use of 4,4'-N,N,N',N'-tetrakis(2-hydroxyethyl)dianiline sulfide (7.4 mmol, 2.90 g) prepared in Example 12, the procedure of Example 4 was repeated to give 1.24 g of the crude product as a light brown oil. The crude product was applied to a silica gel column (40 μm, 5 x 12.5 cm) packed dry. Then it was wet and eluted with hexane / ethyl acetate (5:1) under a positive pressure (15 psi). The collected corresponding fractions were dried over anhydrous sodium sulfate and evaporated to obtain 0.82 g (yield: 23.7%) of the product as yellow oil.

TLC : $R_f$ = 0.51 [hexane/ ethyl acetate (5:1)], UV quenching and NBP positive

$^1H$ NMR ($CDCl_3$) δ (ppm)

6.48-6.80 & 7.10-7.51 [dd, 8H, $S(C_6H_4)_2$],

3.46-3.89 [m, 16H, $N(CH_2CH_2Cl)_2$]

Elemental analysis $C_{20}H_{24}Cl_4N_2S$

Calcd. : C, 51.52; H, 5.19; N, 6.01; S, 6.88; Cl, 30.41

Found : C, 51.43; H, 5.16; N, 5.99; S, 6.92; Cl, 30.37

<u>Example 14 : 4,5'-N,N,N',N'-Tetrakis(2-chloroethyl)dianiline sulfoxide</u>

With the use of 4,4'-N,N,N',N'-tetrakis(2-chloroethyl)dianiline sulfide (0.73 mmol, 0.35 g) prepared in Example 13, the procedure of Example 5 was repeated to give 0.31 g of the crude product as grey viscous oil. The crude oil was diluted in ethyl acetate (2 ml) and applied to three preparative TLC plates, and was then eluted with ethyl acetate / hexane (3:2). The regions with $R_f$ = 0.35 were scraped, dissolved in ethyl acetate / ethanol (1:1), filtered and evaporated to give 220 mg (yield : 60.8%) of the product as a light grey solid.

TLC : $R_f$ = 0.34 [hexane/ ethyl acetate (2:3)]

$^1H$ NMR ($CDCl_3$) δ (ppm)

6.41-6.90 & 7.29-7.67 [dd, 8H, $S(C_6H_4)_2$], 3.55-4.02 [m, 16H, $N(CH_2CH_2Cl)_2$]

Elemental analysis $C_{20}H_{24}Cl_4N_2SO$

Calcd. : C, 49.81; H, 5.02; N, 5.81; S, 6.65; Cl, 29.40

Found : C, 49.83; H, 4.98; N, 5.77; S, 6.72; Cl, 29.34

Example 15 : 4,4'-N,N'-bis(2-hydroxyethyl)dianiline sulfide

4,4'-Diaminodiphenyl sulfide (5.00 g, 23.1 mmol) prepared in Example 11 and 2-chloroethanol (6.2 ml, 7.44 g, 92.4 mmol) were added to a suspension of $CaCO_3$ (4.10 g, 41.0 mmol) in water (150 ml). The cloudy mixture was heated under reflux, while being protected from light for 24 h. An additional 2 equivalents of 2-chloroethanol (3.1 ml, 3.72 g, 46.2 mmol) and $CaCO_3$ (4.62 g, 46.2 mmol) were added and the reaction mixture was heated under reflux for 24 h. The reaction mixture was then cooled, and the pH (4.2) was adjusted to 7.0 with 10% NaOH. The mixture was then extracted with ethyl acetate (4 x 100 ml). The combined organic layer was dried over anhydrous sodium sulfate and evaporated to give 6.23 g of brown oil which contained 7 substances detected by UV after TLC operation. The crude oil was then applied to a silica gel column (40 $\mu$m, 5 x 15 cm) packed dry. Then it was wet and eluted with ethyl acetate / hexane (5:1) under a positive pressure (15 psi). The collected corresponding fractions were dried over anhydrous sodium sulfate and evaporated to obtain 0.77 g (yield: 11.0%) of the product as pale yellow oil.

TLC : $R_f$ = 0.23 (ethyl acetate / hexane = 4:1)

$^1H$ NMR ($CDCl_3$) $\delta$ (ppm)

6.37-6.70 & 6.92-7.31 [dd, 8H, $S(C_6H_4)_2$], 3.76 [s, 2H, $(NH-)_2$], 2.92-3.45 [m, 10H, $N(CH_2CH_2OH)_2$]

Example 16 : 4,4'-N,N'-Bis(2-chloroethyl)dianiline sulfide

With the use of 4,4'-N,N'-bis(2-hydroxyethyl)dianiline sulfide (21.4 mmol, 0.75 g) prepared in Example 15, the procedure of Example 12 was repeated to give 0.63 g of the crude product as golden yellow oil. The crude oil was diluted in ethyl acetate (3 ml) and applied to three preparative TLC plates, and was then eluted with ethyl acetate / hexane (1:3). The regions with $R_f$ = 0.43 were scraped, dissolved in ethyl acetate / ethanol (1:1), filtered and evaporated to give 0.46 g (yield : 52.3%) of the product as a charcoal colored oil.

TLC : $R_f$ = 0.43 [hexane/ ethyl acetate (3:1)], UV quenching and NBP positive

$^1$H NMR ($CDCl_3$) $\delta$ (ppm)

6.51-6.89 & 7.04-7.41 [dd, 8H, $S(C_6H_4)_2$], 3.96 [s, 2H, $(NH-)_2$], 3.18-3.68 [m, 8H, $N(CH_2CH_2Cl)_2$]

Elemental analysis $C_{16}H_{18}Cl_2N_2S$

Calcd. : C, 56.31; H, 5.32; N, 8.21; S, 9.39; Cl, 20.78

Found : C, 56.22; H, 5.28; N, 8.14; S, 9.40; Cl, 20.81

Example 17 : 4,4'-N,N'-Bis(2-chloroethyl)dianiline sulfoxide

With the use of 4,4'-N,N'-bis(2-chloroethyl)dianiline sulfide (0.91 mmol, 0.31 g) prepared in Example 16, 0.29 g of the crude product was obtained as light grey viscous oil. The crude oil was diluted in ethyl acetate (2 ml) and applied to three preparative TLC plates, and was then eluted with ethyl acetate / hexane (1:1). The regions with $R_f$ = 0.35 were scraped, dissolved in ethyl acetate / ethanol (1:1), filtered and evaporated to give 0.23 g (yield : 70.1%) of the product as a white crystalline solid.

TLC : $R_f$ = 0.35 [hexane/ ethyl acetate (1:1)]

$^1$H NMR ($CDCl_3$) $\delta$ (ppm)

6.58-6.98 & 7.16-7.51 [dd, 8H, $S(C_6H_4)_2$], 4.01 [s, 2H, $(NH-)_2$], 3.22-3.75 [m, 8H, $N(CH_2CH_2Cl)_2$]

Elemental analysis $C_{16}H_{18}Cl_2N_2SO$

Calcd. : C, 53.79; H, 5.08; N, 7.84; S, 8.97; Cl, 19.85

Found : C, 53.71; H, 4.97; N, 7.77; S, 8.95; Cl, 19.80

Example 18 : 4-[N,N-Bis(2-hydroxyethyl)]amino-4'-nitrodiphenyl sulfide

To a solution of 4-amino-4'-nitrodiphenyl sulfide (25.00 g, 101.5 mmol) in 200 ml of THF and 250 ml of AcOH, 45 ml of 10% (w/v) of ethylene oxide in THF (102.2 mmol) was added at 5°C. The reaction mixture was stirred at room temperature for several days during which 45 ml of the ethylene oxide solution was added every day until mole ratio of ethylene oxide to the starting compound was about 7. The mixture was then neutralized with aqueous $Na_2CO_3$, and extracted with ethyl acetate three times. The ethyl acetate extract was washed with water, dried over anhydrous sodium sulfate, and evaporated in vacuo to remove the solvent. The crude product was treated with charcoal in ethyl acetate solution to give 19.5 g of yellow product. The residue after evaporation of mother liquid was purified on a flash column using methylene chloride / acetone (8:2) as the eluant to obtain 7.8 g of the product. [Total yield : 80.43%]

m.p. : 99.5 - 100.5 °C

TLC : $R_f$ = 0.41 [methylene chloride / acetone (8:2)]

$^1H$ NMR ($CDCl_3$) δ (ppm)

6.63-8.03 (m, d, 8H, $O_2NC_6H_4SC_6H_4N$), 3.63-3.90 [dd, 8H, $N(CH_2CH_2OH)_2$]

Elemental analysis $C_{16}H_{18}N_2O_4S$

Calcd. : C, 57.47; H, 5.42; N, 8.38; S, 9.59

Found : C, 57.62; H, 5.51; N, 8.30; S, 9.48

Example 19 : 4-[N,N-Bis(2-chloroethyl)]amino-4'-nitrodiphenyl sulfide

A mixture of 10.00 g (29.91 mmol) of 4-[N,N-bis(2-hydroxyethyl)]amino-4'-nitrodiphenyl sulfide prepared in Example 18 and 65 ml of $POCl_3$ was heated under reflux for 1 h, then the hot reaction mixture was poured to chopped ice with stirring. The resultant mixture was extracted with methylene chloride. The extract was treated with activated charcoal, washed with saturated aqueous sodium bicarbonate to pH 6 and washed with water, and dried over anhydrous $Na_2SO_4$, then evaporated in vacuo to give the crude product. This crude product was purified with activated charcoal and crystallized with ethyl acetate / hexane to give 8.90 g of yellow product (yield: 80.15%).

m.p. : 112.5 - 113.5 °C

TLC : $R_f$ = 0.51 [hexane / ethyl acetate (5:1)]

$^1H$ NMR ($CDCl_3$) $\delta$ (ppm)

6.63-8.06 (m, d, 8H, $O_2NC_6H_4SC_6H_4N$), 3.73 [s, 8H, $N(CH_2CH_2Cl)_2$]

Elemental analysis $C_{16}H_{16}N_2O_2Cl_2S$

Calcd. : C, 51.76; H, 4.34; N, 7.55; Cl, 19.10; S, 8.64

Found : C, 51.87; H, 4.31; N, 7.59; Cl, 18.98; S, 8.69

Example 20 : 4[N,N-Bis(2-chloroethyl)]amino-4'-nitrodiphenyl sulfoxide

To a solution of 4-[N,N-bis(2-chloroethyl)]amino-4'-nitrodiphenyl sulfide (13.50 g, 36.36 mmol) in trifluoroacetic acid (TFA, 64 ml), 15.9 ml of $H_2O_2$ / TFA (36.36 mmol, prepared by mixing 4.3 ml of 30% $H_2O_2$ with 14.1 ml of TFA) was added while stirring at 5°C. The reaction was allowed to proceed for 6.5 h. After adding ethyl acetate and water, the reaction mixture was neutralized to

pH 6-7 with aqueous sodium carbonate. The separated aqueous layer was extracted with ethyl acetate three times. Ethyl acetate extracts were combined and washed with water, dried over anhydrous sodium sulfate, and evaporated in vacuo. The crude product was crystallized with ethyl acetate / hexane to give 13.67 g (yield : 97.08%) of a yellow product.

m.p. : 107 - 108 °C

TLC : $R_f$ = 0.45 [hexane / ethyl acetate (1:1)]

$^1H$ NMR ($CDCl_3$) $\delta$ (ppm)

6.63-8.40 (d, m, and d, 8H, $O_2NC_6H_4SOC_6H_4N$),

3.60-3.77 [t, 8H, $N(CH_2CH_2Cl)_2$]

Elemental analysis $C_{16}H_{16}N_2O_3Cl_2S$

Calcd. : C, 49.62; H, 4.16; N, 7.23; Cl, 18.31; S, 8.28

Found : C, 49.77; H, 4.19; N, 7.17; Cl, 18.30; S, 8.21

<u>Example 21 : 4-[N,N-Bis(2-chloroethyl)]amino-4'-aminodiphenyl sulfoxide</u>

Iron (19.14 g, 100 mesh) was activated by refluxing it with 1.5 ml of distilled water and 1 drop of concentrated hydrochloric acid for 0.5 h. Ethanol (68.4 ml) and 4 - [N,N - bis (2 - chloroethyl) amino - 4' - nitrodiphenyl sulfoxide (13.67 g, 35.30 mmol) prepared in Example 20 were added into the mixture at 40 - 50 °C. The reaction mixture was heated under reflux for 15 min. Several drops of 10% NaOH was added to the reaction mixture to pH 8. The hot reaction mixture was then filtered, and the residue was washed with ethanol and water. The filtrate was evaporated in vacuo to remove ethanol, and extracted with ethyl acetate three times. The ethyl acetate extract was dried over anhydrous sodium sulfate and evaporated in vacuo. The crude product was purified on a flash column using ethyl acetate / methylene chloride (6:4) as an eluant, and then

recrystallized from ethyl acetate / hexane to give 9.09 g of off-white crystalline title compound (yield : 72.07%) and 0.8 g (6.64%) of off-white solid identified as 4-[N,N-bis(2-chloroethyl)]amino-4'-aminodiphenyl sulfide.

4-[N,N-bis(2-chloroethyl)]amino-4'-aminodiphenyl sulfoxide

m.p. : 136.5 - 138 °C

TLC : $R_f$ = 0.49 (ethyl acetate)

$^1$H NMR ($CDCl_3$) $\delta$ (ppm)

6.60-7.50 (d, m, 8H, $NC_6H_4SOC_6H_4N$), 3.60-3.73 [t, 8H, $N(CH_2CH_2Cl)_2$]

Elemental analysis $C_{16}H_{18}N_2OCl_2S$

Calcd. : C, 53.79; H, 5.08; N, 7.84; Cl, 19.85; S, 8.97

Found : C, 53.88; H, 5.10; N, 7.89; Cl, 19.90; S, 9.03

<u>4-[N,N-Bis(2-chloroethyl)]amino-4'-aminodiphenyl sulfide</u>

m.p. : 77 - 79 °C

TLC : $R_f$ = 0.64 [methylene chloride / acetone (196:4)]

$^1$H NMR ($CDCl_3$) $\delta$ (ppm)

6.50-7.30 (d, d, 8H, $NC_6H_4SC_6H_4N$), 3.68 [s, 8H, $N(CH_2CH_2Cl)_2$]

Elemental analysis $C_{16}H_{18}N_2Cl_2S$

Calcd. : C, 56.13; H, 5.32; N, 8.21; Cl, 20.78; S, 9.39

Found : C, 56.30; H, 5.33; N, 8.17; Cl, 20.68; S, 9.42

<u>Example 22 : 4-N,N-Bis(chloroethyl)amino-4'-(9-acridinyl)aminodiphenyl sulfide hydrochloride</u>

1) 9-Chloroacridine

A mixture of 5.00 g (5.12 mmol) of 9(10H)-acridone, 25.0 ml of $SOCl_2$ and 0.1 ml of dimethylformamide was heated under reflux until a clear solution resulted and for

additional 0.5 h. After removal of $SOCl_2$ in vacuo, the residue was dissolved in 70 ml of $CHCl_3$ and then stirred into chopped ice. Saturated aqueous $NaHCO_3$ was added to adjust the pH value to 7. The aqueous layer was extracted with $CHCl_3$. Then the combined $CHCl_3$ extract was washed with water, dried over anhydrous magnesium sulfate, and evaporated in vacuo. The residue was crystallized from $CCl_4$ to give 3.1 g (yield: 73.09%) of the product as greenish crystals.

m.p. : 118.5 - 120.5 °C

TLC : $R_f$ = 0.67 [methylene chloride / ethyl acetate (92:8)]

IR showed disappearance of the peak of C=O and appearance of the peak of C-Cl.

2) 4- N,N-Bis(chloroethyl)amino-4′-(9-acridinyl) aminodiphenyl sulfide hydrochloride

A solution of 4-[N,N-bis(2-chloroethyl)]amino-4′-aminodiphenyl sulfide (0.52 g, 1.53 mmol) prepared in Example 21 and 9-chloroacridine (0.30 g, 1.40 mmol) prepared as above in N-methyl-2-pyrrolidinone (13.5 ml) was treated with a few drops of concentrated HCl and then stirred at room temperature for 1.5 h. The mixture was diluted with ethyl acetate, and the resultant precipitate was collected and washed with the same solvent to give a crude product. The crude product was mixed with 10 ml of N-methyl-2-pyrrolidinone and the mixture stirred for 1 h. Ethyl acetate (250 ml) was gradually added to complete the precipitation, and the resultant precipitate was collected and washed with ethyl acetate to give the pure title compound (0.70 g, yield: 89.4%) as reddish solid.

m.p. : 261-262 °C

TLC : $R_f$=0.73 [hexane/EtOAc(1:1)]

$^1H$ NMR ($CDCl_3$ / DMSO-$d_6$ 1:4) $\delta$ (ppm)

6.70-8.40 (m, m, m, 16H, ArH), 3.75 [s, 8H,

$N(CH_2CH_2Cl)_2]$

Elemental analysis $C_{29}H_{26}N_3Cl_3S$

Calcd. : C, 62.76; H, 4.72; N, 7.57; Cl, 19.17; S, 5.78

Found : C, 62.83; H, 4.74; N, 7.48; Cl, 19.06; S, 5.86

Example 23 : 4-[N,N-Bis(chloroethyl)]amino-4'-(9-acridinyl) aminodiphenyl sulfoxide hydrochloride

By the use of 4-[N,N-bis(2-chloroethyl)]amino-4'-aminodiphenyl sulfoxide (1.00 g, 2.81 mmol) prepared in Example 21 and 9-chloroacridine (0.55 g, 2.57 mmol) prepared in Example 22, the procedure of Example 22 2) was repeated to give 1.3 g (yield: 88.45%) of the title compound as yellow solid.

m.p. : 250-251 °C

TLC : $R_f$ = 0.81 (ethyl acetate)

$^1H$ NMR ($CDCl_3$ / DMSO-$d_6$ 1:4) $\delta$ (ppm)

6.75-8.40 (m, m, m, 16H, ArH), 3.75 [s, 8H, $N(CH_2CH_2Cl)_2]$

Elemental analysis $C_{29}H_{26}N_3Cl_3OS$

Calcd. : C, 61.01; H, 4.59; N, 7.36; Cl, 18.63; S, 5.62

Found : C, 60.84; H, 4.86; N, 7.34; Cl, 18.47; S, 5.53

Experimental Example 1 : In Vitro Cytotoxicity Assessment

A confluent flask of Chinese Hamster Lung V-79 transformed cells was scraped to collect the monolayer and the cells were counted by trypan blue dye exclusion. Glass milk dilution bottles were then inoculated with 500,000 cells in 10 ml of medium (minimum essential medium eagle supplemented with 10% fetal bovine serum, Penicilline G sodium, streptomycin sulfate, amphotericin and essential and non-essential amino acids and vitamins) and incubated

for 20 hours in a 95% air/ 5% humidified atmosphere. One set of six bottles was sealed with sterilized rubber septa and fitted with 21-gauge needles for gas inflow and outflow. Each bottle was gassed individually, all outlet tubings and additive tubings immersed in water were connected to a flow meter (average flow velocity = 0.15 liter/min) to allow visible monitoring of gas flow as well as to prevent back flow of air into the cultures. To produce hypoxia, cultures were gassed continuously for 2h at 37°C with a humidified mixture of 95% $N_2$ / 5% $CO_2$. Normally aerated cultures were maintained in air until drug treatment. At this time, appropriate dilutions of the drug in ethanol/water were added directly to the cultures, without breaking the hypoxia, by injecting 0.25 ml of drug through the rubber septa. In the aerobic set, cells were exposed to drug for 3h under normal aerated conditions. In hypoxic set, cells were gassed 10 min after injection of the drug, needles were pulled out and the sealed bottles were incubated for 3h in a normally aerated incubator. After drug exposure, the cells were washed twice with 5 ml sterile HBSS, and treated with 2.5% trypsin (Gibco® labs) for 2-3 min. Cells were collected by centrifugation, resuspended in 5 ml fresh solvent/or buffer and counted by trypan blue dye exclusion. Appropriate dilutions were made, and 500 cells were cultured in triplicate in a total of 10 ml medium. Dishes were incubated for 6-8 days to allow colony formation. Colonies were then rinsed twice with 0.9% saline, fixed with ethanol, stained with crystal violet and counted. Results were reported as the number of colonies surviving chemical treatment per number of colonies in the solvent treated control. The $IC_{50}$ values were determined by semi-logarithm plotting the drug concentration versus cell viability as determined by the number of colonies surviving the treatments. The results of the plotting with regard to the compounds prepared in the

Examples are shown in Figs. 1 - 4. The $IC_{50}$ and $IC_{90}$ values under normal or hypoxic condition are shown in Table 1 below.

Table 1

| Ex. No. | $IC_{50}$ (μM/ml) | $IC_{90}$ (μM/ml) | |
|---|---|---|---|
| | normal condition | normal condition | hypoxic condition |
| Example 4 | 2.46 ± 0.14 | | |
| Example 5 | 433.9 ± 23.7 | 939.2 ± 43.4 | 290.7 ± 29.1 |
| Example 9 | 1.31 ± 0.24 | | |
| Example 10 | 524.6 ± 14.3 | 1006.3 ± 65.8 | 231.4 ± 37.5 |
| Example 13 | 0.31 ± 0.20 | | |
| Example 14 | 90.0 ± 0.84 | 417.4 ± 70.7 | 50.5 ± 13.5 |
| Example 16 | 16.3 ± 1.43 | | |
| Example 17 | 670.7 ± 123.2 | | |
| Example 20 | | 3583.6±47.8 | 117.8±21.6 |
| Example 21 | | 4801.3±81.7 | 118.3±25.1 |
| Example 23 | | 3648.5±77.4 | 113.6±19.8 |

As can be shown from the above results, the sulfoxide derivatives according to the present invention have much lower cytotoxicity than the corresponding sulfide. It is found that the sulfoxides have rather higher cytotoxicity under hypoxic condition than under normal condition so that they have selective effects on a solid cancer having hypoxic condition as compared to the normal cells.

Experimental Example 2 : Tumor Inhibition in vivo

The B16 transformed cells cultured in vitro were treated with 0.25% trypsin, collected, washed with sterilized phosphate buffered saline (PBS). And then the cell density was adjusted to $10^7$ cells/ml. After 0.2 ml of

cells (2 x $10^6$ cells/ 0.2 ml/ mouse) were implanted into the subcutaneous tissue of the shoulder's upper part of BDF1 male mouse whose fur was previously eliminated, they were passaged in vivo per 14 days. B16 tumors were carefully isolated from the BDF1 male mouse tissue that had been passaged, necrosis cite was removed, and the weight of fresh tumor was measured. After adding sterilized physiological saline solution of 10-folds of the tumor weight, the mixture was ground by using a glass homogenizer to prepare a supernatant. The supernatant was transplanted to a subcutaneous tissue of the shoulder's upper part of BDF1 male mouse (0.2 ml/ mouse) (0th day). After 8 days, 45 animals having tumor tissue of 100 - 300 $mm^3$ volume were selected and divided into the experimental groups at random (6 animals per each group). The experimental compound(s) was administered into the abdominal cavity at the day 8, 12, 16, and 20. As the experimental compound, 200 mg/kg of cyclophosphamide and 60 mg/kg of 2-hydroxypyrimidine were firstly administered into the abdominal cavity, and after 1 hr the compound according to the present invention(300, 150 mg/Kg) was administered. The tumor inhibition effect was evaluated by the body weight and the tumor growth inhibition rate (TGI) determined by the volumes of tumor in administered group and cotrol group at the day 8, 12, 16 and 20.

* Volume of tumor ($mm^3$) = a x b x b x 1/2

    a : the longest diameter of the tumor

    b : the shortest diameter of the tumor

* TGI (Tumor Growth Inhibition rate, %) = (1 - Vt / Vc) x 100

    Vt : tumor volume of the administered group ($mm^3$)

    Vc : tumor volume of the vehicle control group ($mm^3$)

The results of the plotting with regard to the

compounds prepared in Examples are shown in Figs. 5 and 6. The change of body weight of the animals and tumor growth inhibition rate are shown in Tables 2 and 3 below.

Table 2 : Change of body weight of the $BDF_1$ mice transplanted with B16 tumor tissue

| Example | dose (mg/kg) | No. of animals | Change of body weight (g) | | | | |
|---|---|---|---|---|---|---|---|
| | | | 8th day | 12th day | 16th day | 20th day | 24th day |
| control | (40% PEG 400 in saline) | 8 | 18.45 ± 0.28 | 21.18 ± 0.26 | 21.77 ± 0.31 | 22.30 ± 0.30 | 24.49 ± 0.62 |
| CPM | 200 | 7 | 19.73 ± 0.47 | 21.01 ± 0.50 | 20.96 ± 0.62 | 20.69 ± 0.95 | 21.16 ± 0.73** |
| Example 23, CPM, 2-hydroxypyrimidine | 300 200 60 | 6 | 18.53 ± 0.64 | 19.88 ± 0.73 | 20.36 ± 0.87 | 19.97 ± 0.52** | 20.06 ± 0.54** |
| Example 23, CPM, 2-hydroxypyrimidine | 150 200 60 | 6 | 19.11 ± 0.90 | 20.43 ± 0.96 | 20.41 ± 0.83 | 21.54 ± 0.77 | 21.29 ± 1.18* |
| Example 23, 2-hydroxypyrimidine | 300 60 | 6 | 19.47 ± 0.66 | 21.29 ± 0.58 | 21.61 ± 0.67 | 21.92 ± 0.68 | 23.24 ± 0.47 |
| Example 21, CPM, 2-hydroxypyrimidine | 300 200 60 | 6 | 18.80 ±0.64 | 19.80 ± 0.73 | 19.58 ± 0.94* | 20.08 ± 0.97* | 20.07 ± 0.60** |
| Example 21, CPM, 2-hydroxypyrimidine | 150 200 60 | 6 | 18.61 ± 0.66 | 19.94 ± 0.64 | 19.96 ± 0.81* | 20.82 ± 0.80 | 21.37 ± 0.91* |

(* : P<0.05, ** : P<0.01 statistically significant.

CPM : cyclophosphamide)

Table 3 : Results of tumor growth inhibition test in $BDF_1$ mice transplanted with B16 tumor tissue

| Example | dose (mg/kg) | No. of animals | Change of tumor volume ($mm^3$) 8th day | 13th day | 15th day | 18th day | 21th day |
|---|---|---|---|---|---|---|---|
| control | (40% PEG 400 in saline) | 8 | 195.7 ± 41.7 | 1954.2 ± 280.1 | 3206.4 ± 392.8 | 5893.2 ± 676.9 | 10150 ± 1023 |
| | | | - | 100%(0) | 100%(0) | 100%(0) | 100%(0) |
| CPM | 200 | 7 | 185.9 ± 27.8 | 896.9 ± 222.3* | 1414.3 ± 212.9** | 1937.1 ± 224.9** | 2844 ± 326** |
| | | | - | 45.9(54.1) | 44.1(55.9) | 32.9(67.1) | 28.0(72.0) |
| Example 23, CPM, 2-hydroxypyrimidine | 300<br>200<br>60 | 6 | 153.4 ± 31.9 | 638.5 ± 128.2** | 1075.2 ± 200.9** | 1690.7 ± 245.1** | 2105 ± 327** |
| | | | - | 32.7(67.3) | 33.5(66.5) | 28.7(71.3) | 20.7(79.3) |
| Example 23, CPM, 2-hydroxypyrimidine | 150<br>200<br>60 | 6 | 118.6 ± 29.7 | 514.4 ± 164.6** | 1019.3 ± 237.3** | 1460.5 ± 245.1** | 2268 ± 310** |
| | | | - | 26.3(73.7) | 31.8(68.2) | 24.8(75.2) | 22.3(77.7) |
| Example 23, 2-hydroxypyrimidine | 300<br>60 | 6 | 114.9 ± 25.4 | 806.2 ± 225.4* | 1667.8 ± 309.9* | 2922.0 ± 588.9* | 5408 ± 804** |
| | | | - | 41.3(58.7) | 52.0(48.0) | 49.6(50.4) | 53.3(46.7) |
| Example 21, CPM, 2-hydroxypyrimidine | 300<br>200<br>60 | 6 | 157.4 ± 41.6 | 680.9 ± 195.7** | 1109.7 ± 282.3** | 1335.0 ± 331.5** | 1959 ± 405** |
| | | | - | 34.8(65.2) | 34.6(65.4) | 22.7(77.3) | 19.3(80.7) |
| Example 21, CPM, 2-hydroxypyrimidine | 150<br>200<br>60 | 6 | 151.1 ± 20.5 | 675.3 ± 189.7** | 1320.7 ± 269.1** | 1801.7 ± 194.0** | 2597 ± 278** |
| | | | - | 34.6(65.4) | 41.2(58.8) | 30.6(69.4) | 25.6(74.4) |

(* : $P<0.05$, ** : $P<0.01$ statistically significant

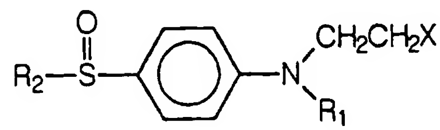
CPM : cyclophosphamide)

As can be shown from the results, the growth of tumor can be inhibited by about 80% in case of administrating the

sulfoxides of the present invention. The tumor inhibition effect rises by 10% when using the compound of the present invention with the conventional compounds as compared to the case using only cyclo phosphamide (70%), which whows the inhibition of growth of the solid cancer transplanted to mice.

**What is claimed is :**

1. Sulfoxide derivatives of nitrogen-mustard represented by a general formula(I) and pharmaceutically acceptable salts thereof :



(I)

wherein, $R_1$ represents a hydrogen, or haloethyl; $R_2$ represents a lower alkyl or phenyl group substituted or non-substituted; and X represents a halogen atom, provided that $R_1$ is a chloroethyl and X is a chlorine, $R_2$ does not represent methyl group.

2. Sulfoxide derivatives and salts thereof according to claim 1, wherein the compound is p-(n-propylsulfinyl)-N,N-bis(2-chloroethyl)aniline.

3. Sulfoxide derivatives and salts thereof according to claim 1, wherein the compound is p-(phenylsulfinyl)-N,N-bis (2-chloroethyl)aniline.

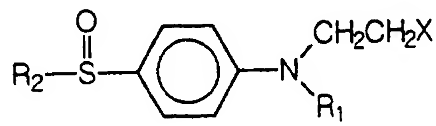
4. Sulfoxide derivatives and salts thereof according to claim 1, wherein the compound is 4,4'-N,N,N',N'-tetrakis(2-chloroethyl)dianiline sulfoxide.

5. Sulfoxide derivatives and salts thereof according to claim 1, wherein the compound is 4,4'-N,N'-bis(2-chloroethyl)dianiline sulfoxide.

6. Sulfoxide derivatives and salts thereof according to claim 1, wherein the compound is 4-[N,N-bis(chloroethyl)]

amino-4'-(9-acridinyl) aminodiphenyl sulfoxide.

7. An anticancer agent comprising sulfoxide derivatives of nitrogen-mustard represented by a general formula(I) and pharmaceutically acceptable salts thereof as an active ingredient, with pharmaceutically acceptable carrier(s) :



(I)

wherein, $R_1$ represents a hydrogen, or haloethyl; $R_2$ represents a lower alkyl or phenyl group substituted or non-substituted; and X represents a halogen atom, provided that $R_1$ is a chloroethyl and X is a chlorine, $R_2$ does not represent methyl group.

Fig.1. Survival of V-79 cells with compound (2) under normal and hypoxic conditions.

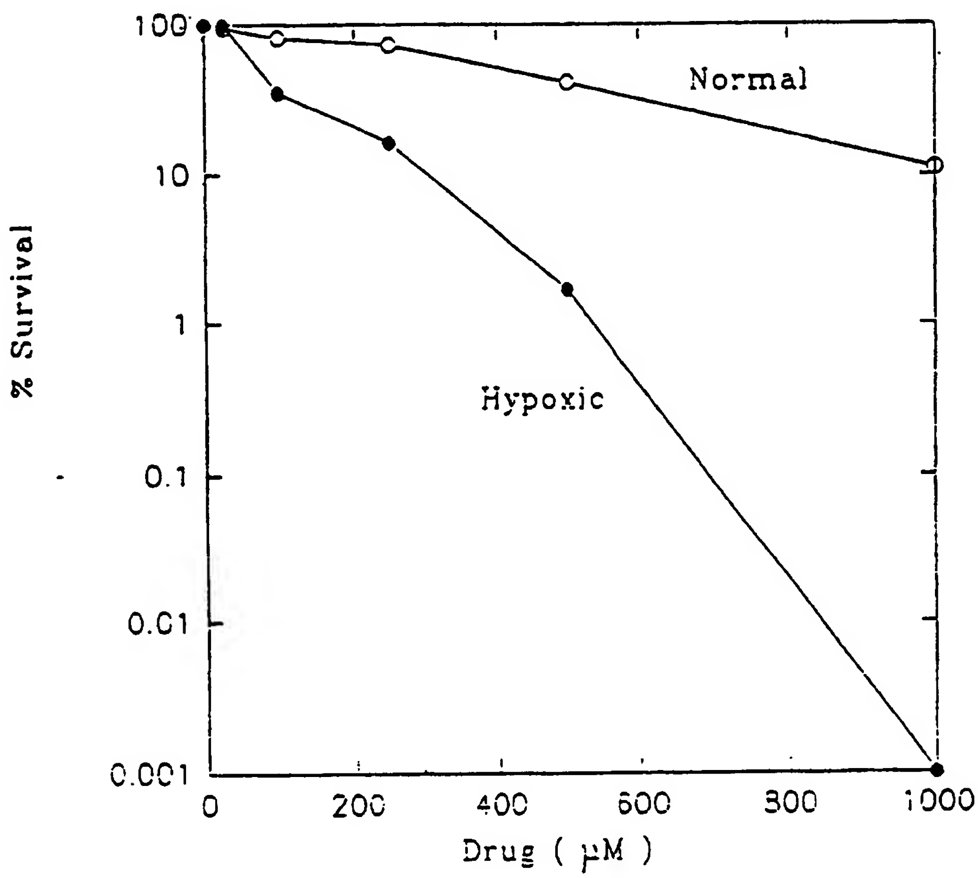


Fig.2. Survival of V-79 cells with compound (4) under normal and hypoxic conditions.

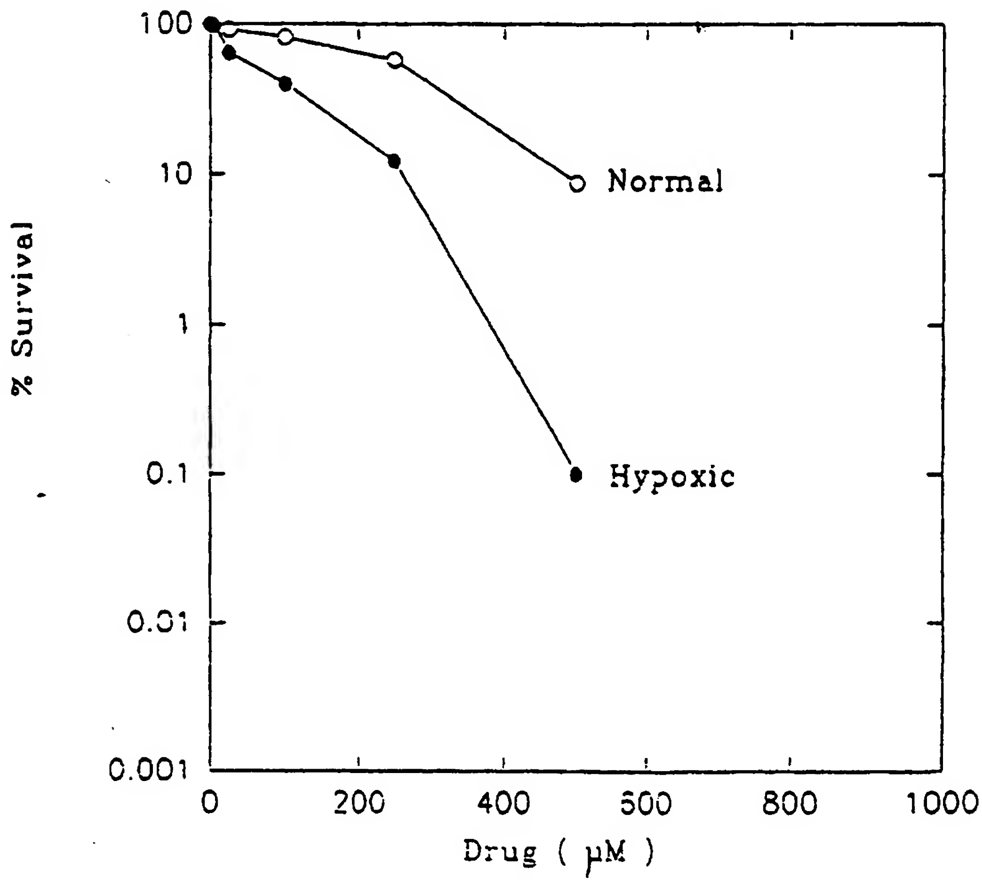


Fig.3. Survival of V-79 cells with compound (6) under normal and hypoxic conditions.

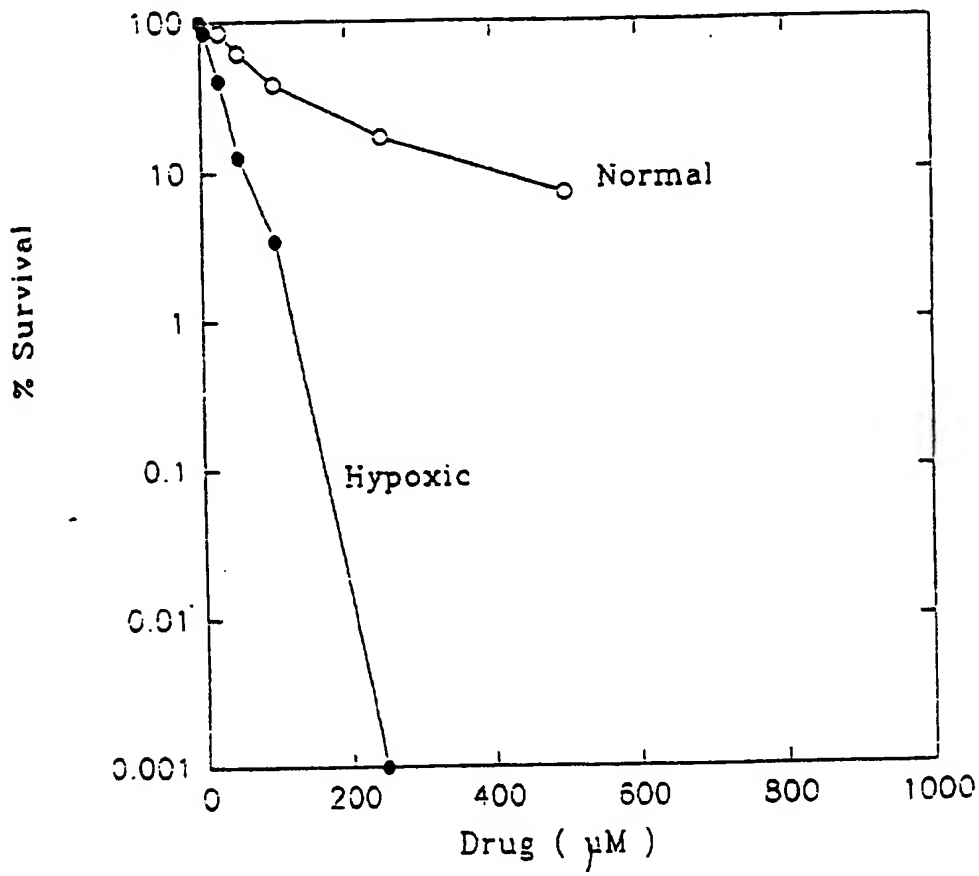


Fig.4. Survival of V-79 cells with compound (6) under normal and hypoxic conditions.

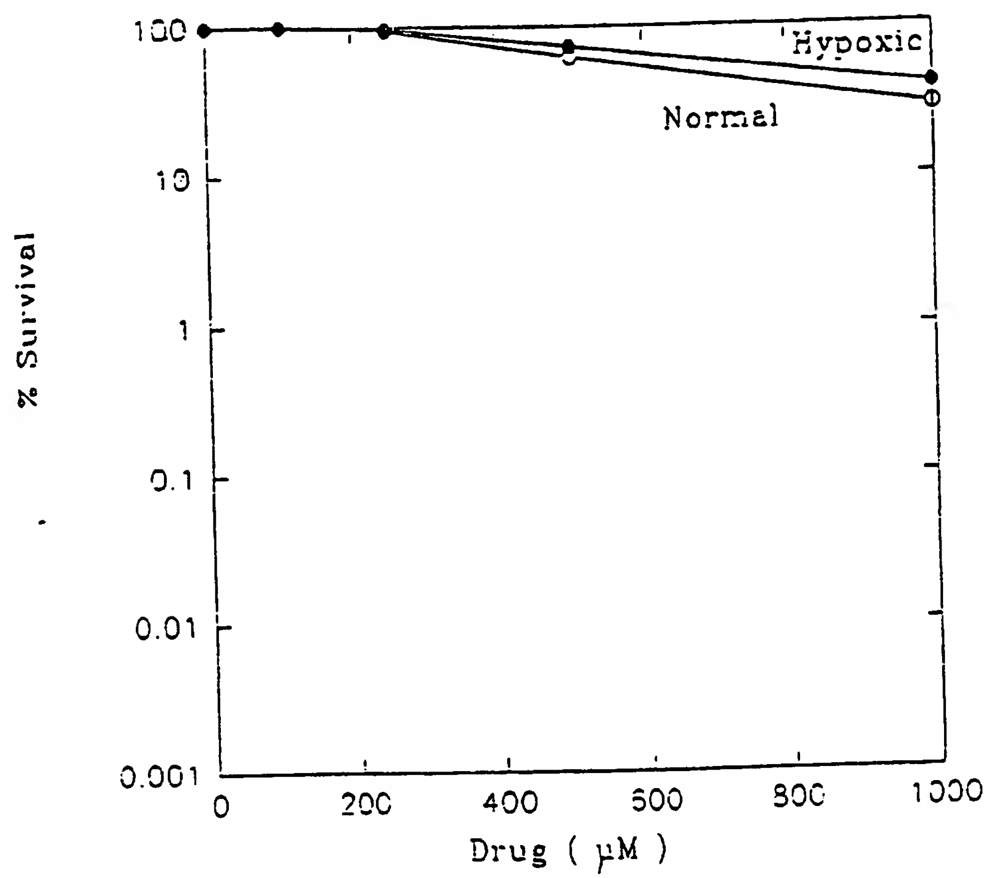


Fig 5. Change of body weight of the BDF1 mice transplar with B16 tumor cells.

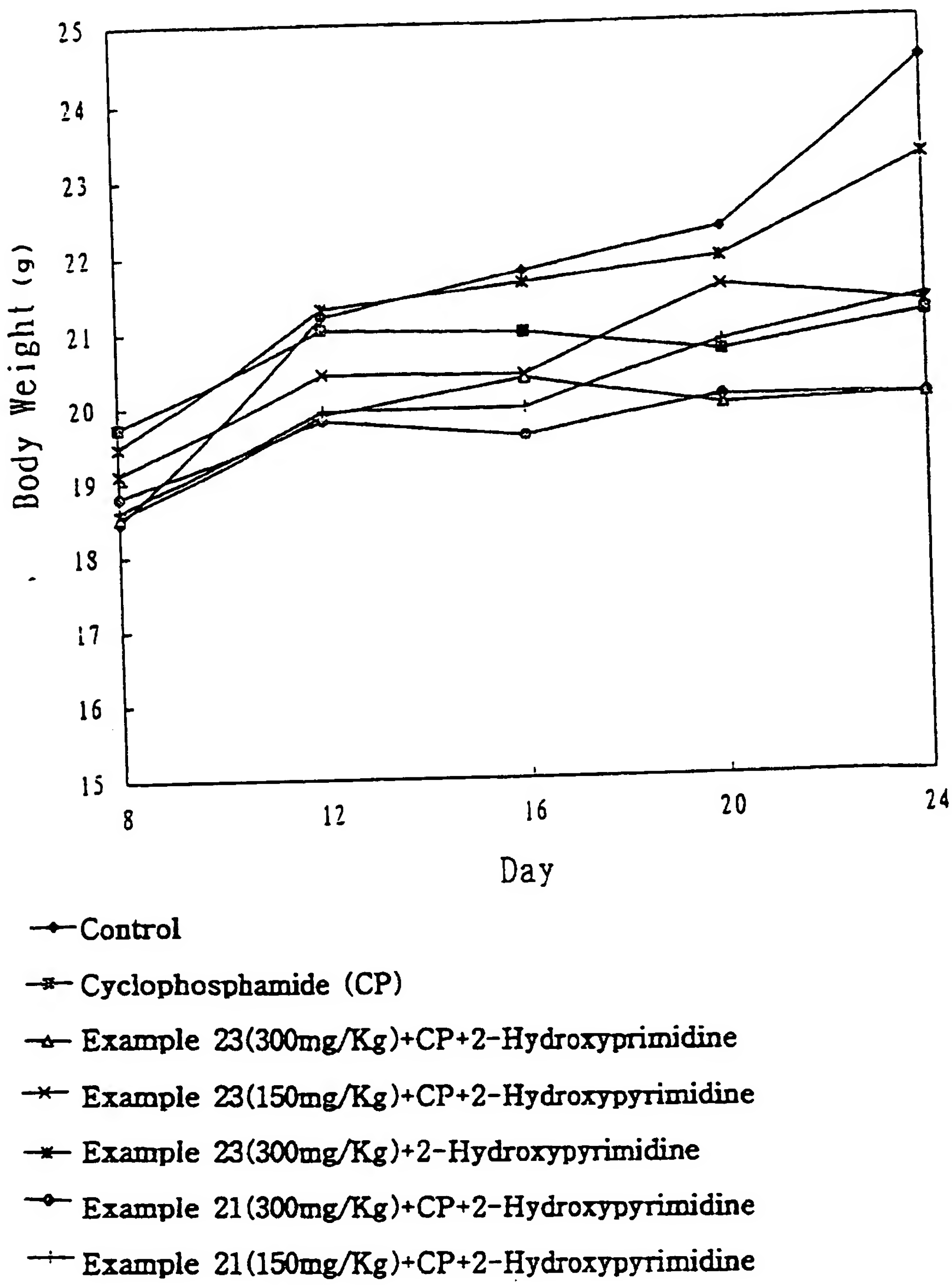
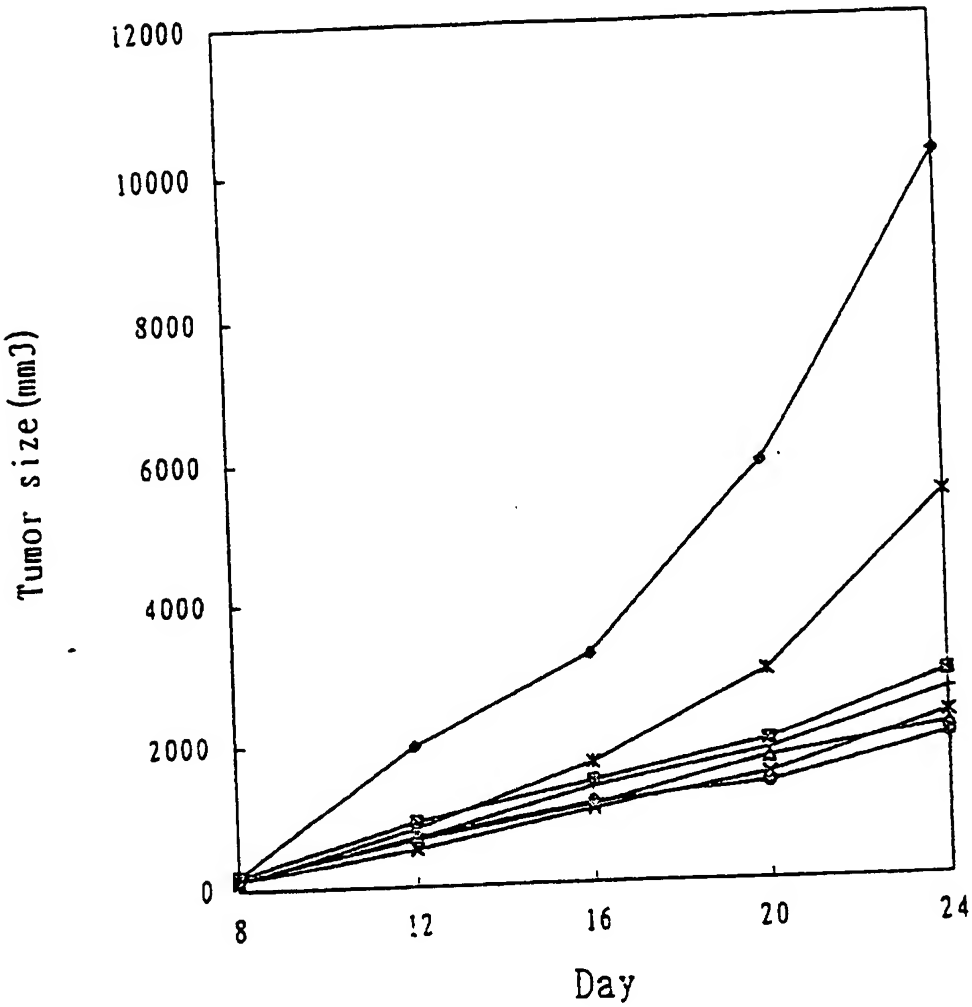


Fig 6. Results of tumor growth inhibition test in BDF1 transplanted with B16 tumor cells.




—◆— Control

—✱— Cyclophosphamide (CP)

—▲— Example 23(300mg/Kg)+CP+2-Hydroxypyrimidine

—×— Example 23(150mg/Kg)+CP+2-Hydroxypyrimidine

—✱— Example 23(300mg/Kg)+2-Hydroxypyrimidine

—●— Example 21(300mg/Kg)+CP+2-Hydroxypyrimidine

—+— Example 21(150mg/Kg)+CP+2-Hydroxypyrimidine

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR 96/00173

**A. CLASSIFICATION OF SUBJECT MATTER**

$IPC^6$: C 07 C 317/36; C 07 D 219/10; A 61 K 31/135

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

$IPC^6$: C 07 C 317/00; C 07 D 219/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

AT

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPIL

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | Chemical Abstracts, Vol.117, No.5, 03 August 1992 (Columbus, Ohio, USA), page 32, column 1, abstract No.117:3991g, KWONG, CHUL HOON et al.:"p-(Methyl=sulfinyl)phenyl nitrogen mustard as a novel bioreductive prodrug selective against hypoxic tumors", & J.Med.Chem. 1991, 35(11), 2137-9. | 1-5,7 |
| A | Chemical Abstracts, Vol.114, No.23, 10 June 1991 (Columbus, Ohio, USA), page 816, column 1, abstract No.114:228692k, GRAVATT, G. LANCE et al.:"DNA-directed alkylating agents. 4. 4-Anilinoquinoline-based minor groove directed aniline mustards", & J.Med.Chem. 1991, 34(5), 1552-60. | 1,7 |
| A | Chemical Abstracts, Vol.113, No.25, 17 December 1990 (Columbus, Ohio, USA), page 704, column 1, abstract No.113:231187j, VALU, KISIONE K. et al.:"DNA-directed alkylating agents. 3. Structure-activity relationships for acridine-linked aniline mustards: consequences of varying the length of the linker chain", & J.Med.Chem. 1990, 33(11), 3014-19. ---- | 1,6,7 |

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 21 November 1996 (21.11.96) | 09 December 1996 (09.12.96) |
| Name and mailing address of the ISA/ AT<br>AUSTRIAN PATENT OFFICE<br>Kohlmarkt 8-10<br>A-1014 Vienna<br>Facsimile No. 1/53424/535 | Authorized officer<br>Reif<br>Telephone No. 1/53424/323 |